# Biological criteria for submergence of physostome (Atlantic salmon) and physoclist (Atlantic cod) fish in sea-cages

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**Contents**

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## SUMMARY

This thesis aims to describe behavioural responses and welfare parameters for the physostome Atlantic salmon and physoclist Atlantic cod when out of neutral buoyancy at different degrees and periods of time. The background for this approach is to improve the culture conditions by fully submergence of the farming installations, as the water below 10 m depth often is more stable in terms of environmental factors such as temperature and current in addition to the lack of waves, which again opens for alternative sites in more exposed oceanic areas.

Salmon were submerged in large-scale fully submersible cages at depths between 4 to 15 m for 22 days (Paper I) and 10 to 25 m for 42 days (Paper II) under different light conditions and at various times of year. The behaviour of individual salmon in a school under submerged conditions was studied to reveal whether a range of coping abilities among individuals exists during the new and more challenging conditions (Paper III). Atlantic cod in an experimental submersible cage were raised from five different starting depths (between 30 and 8 m) and lowered from surface position to 10 m, 20 m and 30 m to test a protocol for safe lifting and lowering steps. Based on the behavioural responses, safe acclimation times before the next vertical step at high and low sea temperatures were identified (Paper IV).

In Papers I and II, the general patterns of swimming depth and schooling density were studied at group level using echo-sounders in addition to swimming speed and swimming angle based on instantaneous observations with underwater cameras. Welfare parameters were defined as weight gain, feed intake, feed utilisation and fin and vertebral condition during the experimental period. The behaviour of individual salmon was studied by monitoring the swimming depth and body temperature using data storage tags implanted in randomly selected fish. Whether the development of diel vertical migration (DVM) activity during feeding was linked with individual growth rates was analysed to study individual coping styles. The immediate response and recovery time after lifting and lowering of Atlantic cod were estimated through measurements of swimming speed, swimming tail beat rate and swimming angle based on underwater camera recordings, and additionally by echo-sounder data obtained from resting fish on the net-floor (Paper IV).

Atlantic salmon submerged without the opportunity to refill their swim bladder lost gas steadily over time, and the bladder was empty after about three weeks (Papers I and II). Swimming speeds were elevated on the first day after submergence and schooling became more structured (more constant speed and a greater distance to neighbouring fish). The diurnal vertical swimming pattern for the salmon in spring was broadly similar to that observed prior to submergence, as the artificial underwater lighting allowed the salmon to keep high swimming speed and lift during night (Paper III). In contrast, about 90% of the salmon submerged below 10 m during the dark winter reversed their diurnal pattern from swimming at shallow depths at night and deeper during the day to swimming deep at night and shallower during the day (reversed DVM) or to swimming with a normal or reversed diurnal pattern on different days (irregular). A separation of faster- and slower-growing salmon also gradually appeared, where the faster-growing individuals occupied the deeper part of the water column. This situation occurred also at night at the end of the experiment among the salmon given continuous light (Paper III). Growth was clearly reduced in the salmon that were submerged during the winter, with more injuries recorded on their fins and snout. Slightly compressed vertebrae in the tail region were also observed, probably due to their tilted head-up tail-down swimming angle during the dark nights (Paper II).

Lifting farmed cod from five different start depths, equivalent to a 40% pressure reduction, resulted in strong downwards swimming movements dependent on the water temperature (Paper IV). The depth before cage lifting affected the immediate response, as the fish became more active after lifting events from shallow compared to deeper depths. Appetite decreased after lifting, but loss of behavioural control was never observed. During the subsequent 8-10 hr recovery periods, swimming activity gradually decreased to the same level as before lifting. The overall recovery time did not depend on start depth or temperature. Independent of final depth or temperature, rapid lowering of cod only resulted in a moderate short-term increase in upwards swimming movements, while appetite was less affected than after lifting. A compressed swim bladder after descents from the surface to 10-30 m leads to negative buoyancy, which required 18-90 h to re-fill by gas secretion, which is a temperature-related process (Paper IV).

In conclusion, this thesis demonstrates that air gulping is a behavioural need for Atlantic salmon, and that long term denial of surface access will reduce their welfare.

Atlantic cod cope well in fully submerged cages, but 40% pressure reduction is near the upper limit for lifts of healthy farmed cod. Secondary lifts should not be done until at least 10 h after the first lift. Cage lowering should be done slowly to avoid potentially stressful crowding of negatively buoyant fish on the cage bottom, especially at low temperatures.

## LIST OF PAPERS

**Paper 1.**

Dempster, T., Korsøen, Ø., Folkedal, O., Juell, J.E., Oppedal, F.

Submergence of Atlantic salmon (*Salmo salar*) in sea-cages: a potential short-term solution to poor surface conditions. Aquaculture 288 (2009), 254-263.

**Paper 2.**

Korsøen, Ø.J., Dempster, T., Fjelldal, P.G., Oppedal, F., Kristiansen, T.S.

Long-term culture of Atlantic salmon (*Salmo salar* L.) in submerged cages during winter affects behaviour, growth and condition. Aquaculture 296 (2009), 373-381.

**Paper 3.**

Korsøen, Ø.J., Dempster, T., Oppedal, F., Kristiansen, T.S.

Individual variation in growth and vertical swimming behaviour in Atlantic salmon (*Salmo salar* L.) subjected to submergence in sea-cages. Manuscript.

**Paper 4.**

Korsøen, Ø.J., Dempster, Fosseidengen, J.E., Fernö, A., Heegaard, E. and Kristiansen, T.S.

Behavioural responses to pressure changes in cultured Atlantic cod (*Gadus morhua*): Defining practical limits for submerging and lifting sea-cages. Aquaculture 308 (2010), 106-115.

# INTRODUCTION

## Fish farming in submersible cages

A number of production problems beset the aquaculture production of Atlantic salmon and Atlantic cod. These include escape of farmed fish (Naylor et al. 2005), sea-lice infestations (Heuch et al. 2005), and occasionally unsuitable culture conditions, including high temperatures, low oxygen concentrations (Johansson et al. 2006; Oppedal et al. 2011), high levels of aluminium in surface freshwater inputs (Bjerknes et al. 2003) and harmful algal and jellyfish blooms (Sammes and Greathead 2004). The direct economic costs of these problems to industry are substantial and lead to production inefficiencies. The environmental costs of escaped fish and sea lice infestations have also been suggested to be substantial (Heuch and Mo 2001; Naylor et al. 2005). A common element of these production problems is that sea-based salmon and cod aquaculture is limited to cages held permanently near the surface.

This has led the industry to develop and use very deep nets in standard surface-based farming to enable fish to better adapt their swimming depth to the local environment. The volume of nets has grown from an initial 1000 $m^3$ in the 1980s to > 60 000 $m^3$ today. Larger units have delivered fish production cost advantages to the industry and may have improved fish welfare.

Another option is to fully submerge the installations, which may diminish the severity of many problems (Dempster et al. 2009). If a cage is submerged deeper than 10 m, it would move the fish away from most unfavourable surface conditions such as heavy waves and strong currents (Ryan 2004), sea lice infestation (Johanessen 1978) and fluctuating environmental conditions in surface waters (Oppedal et al. 2011). In addition to an improved environment for the fish, submersible farms could permit alternative sites in more exposed areas to be utilised.

Farming open swim bladder species (see below) such as Atlantic salmon in submersible cages has been tested on several occasions. Motivated by heavy algae blooms in the 1980s, experiments involving the submergence of rainbow trout (*Oncorhynchus mykiss*) and salmon in small cages (4 × 4 × 2 m) resulted in rapid onset of tilted swimming, loss of buoyancy control, and in some cases exhaustion and mortality (Fosseidengen et al. 1982; Ablett et al. 1989). In contrast, trials in large sea-cages (Osland et al. 2001; Dempster et al. 2008)

demonstrated that salmon could cope with submergence, as they feed actively and grow, although at a slower rate than the surface control cages. Submergence of salmon heavier than 2.5 kg (Ablett et al. 1989) has not previously been investigated.

Several marine species with a closed swim bladder have been farmed in submersible cages in many parts of the world; among them are sea-bream and sea-bass in the Mediterranean (Ryan 2004), cobia in the Caribbean (Benetti et al. 2010), and Atlantic cod in Canada (Chambers and Howell 2006; Rillahan et al. 2009). Norwegian Atlantic cod farming is based in many ways on technology developed for salmon production, and standard surface-based cages have been used.

### *Necessary biological criteria for submersible farming*

Life in the water sounds simple for a fish! However, a fish in a fully submersible cage must maintain neutral buoyancy in water at an energetically positive cost-benefit level without losing normal growth performance. Adaptation to ambient changes in water pressure during vertical repositioning of the cage is another critical factor under such farming techniques. The fish must be allowed to utilise its entire anatomically and behaviourally defined repertoire to cope with the farming environment.

## Living under water

### *Pressure and relative pressure changes*

The atmospheric pressure at sea-surface level is close to 1 atm (1.013 25 bar or 760 mm Hg). Assuming the density of sea water to be 1025 kg $m^{-3}$ (in fact it is slightly variable), pressure increases by about 1 atm with each 10 m of depth (e.g. Blaxter 1980); hence the pressure at 10 m will be 2 atm, a 100% increase relative to the pressure at the surface. At 20 m the pressure is 3 atm, an increase relative to the surface of 200%, and so on. A change in depth from 20 m to 10 m will reduce the pressure from 3 atm to 2 atm which is 33% relative to the start depth, and an ascent from 10 m to the surface will reduce the relative pressure by 50%. The relative reduction in pressure thus changes faster closer to the surface (Schmidt-Nielsen 1975).

### *Buoyancy*

Many fish species seem to be weightless in the water when they are hanging motionless in the water column, while others need to move continuously to avoid sinking. According to Archimedes' principle any object, wholly or partly immersed in fluid, is buoyed up by a force equal to the weight of the fluid displaced by the object. This means that any object whose density is greater than that of water will sink. A wide range of adaptations exists to provide lift to neutralise the lack of buoyancy. Swimming, body size and shape, fins and tails, liver size, body-fat content and water content are some of the factors that provide various degrees of lift in water (Alexander 1966); see also Box 1.

***Some strategies to avoid sinking in water*** **Box 1**

- Bottom fish such as flounders are negatively buoyant so as to lay motionless on the bottom, and have no gas-filled swim bladder for buoyancy control (Blaxter and Tytler 1978).
- Some deep sea bathypelagic fish such as the elongated bristlemouth (*Gonostoma*) have an oil-filled swim bladder (Blaxter and Tytler 1978).
- Most demersal and pelagic fish have a closed soft-walled gas bladder (Horn 1975).
- Some pelagic species such as herring (*Clupea harengus*), have a semi-closed gas bladder, with both a pneumatic duct and an opening to the intestine through which expanding gas can be released during a rapid ascent (Blaxter and Tytler 1978).
- Surface-dwelling species such as salmonids have an open gas bladder (Blaxter and Tytler 1978).
- Fast swimmers, such as the mackerels, have no gas bladder at all, and instead swim constantly to maintain buoyancy control. This enables them to be flexible predators (Blaxter and Tytler 1978).
- A variety of other strategies to avoid sinking in water exists, e.g. increasing the proportion of light-weight substances such as fats and oils in the body, or making the organism hypotonic (Schmidt-Nielsen 1975).

Most fish that live in the uppermost 200 metres of the sea have solved the buoyancy problem by means of gas floats, in a soft-walled air bladder (Horn 1975). Gas has a very low density compared with an equal volume of water, and even a small volume of air can be a good way of dealing with the problem of negative buoyancy (Schmidt-Nielsen 1975).

*The function and size of the swim bladder*

The functions of the swim bladder differ from species to species. In this thesis, I focus on the buoyancy function of the swim bladder. In addition to this function it can also act as a storage chamber for oxygen, for the detection of pressure changes, including sounds and as a sound production organ when drumming muscles surround parts of the bladder (Alexander 1966; Blaxter and Tytler 1978; Nordeide et al. 2008).

The magnitude of the buoyant force of any object in water can be calculated as:

$F_b = - \rho_w \cdot V_{disp} \cdot g$

where $\rho_w$ is the density of water, $V_{disp}$ is the volume of the displaced body of liquid, and $g$ is the gravitational acceleration (wikipedia.org/wiki/Buoyancy). The negative sign means the force is opposite of the gravity force which can be calculated as:

$F_g = m \cdot g$

where $m$ is the mass of the object.

From this follows that neutral buoyancy equals $F_g + F_b = 0 \Rightarrow m = \rho_w \cdot V_{disp}$

For example, a fish in seawater with a body mass of 5 kg with an internal 'air balloon' corresponding to 5% of body volume will have a swim bladder volume of around 250 ml, which can provide a lifting power of 1.026 g $ml^{-1} \cdot 250$ ml = 257 g in sea water and 250 g in freshwater. The swim bladder volume is therefore slightly larger in freshwater species (6-9%) than in seawater species (3-6%) (Alexander 1966).

*Advantages and disadvantages of the gas-filled swim bladder*

The swim bladder reduces the metabolic cost of maintaining buoyancy by around 90% compared to hydrodynamic compensation (Alexander 1966), and the muscular energy thus saved can be utilised for forward swimming. Alternatively, the fish can rest and maintain its pelagic position in mid-water by the lift given by the swim bladder volume (Alexander 1966).

One disadvantage of a gas bladder is that it is easily compressed when the pressure rises and expands when it falls. The gas in a soft-walled swim bladder will change volume according to Boyle's Law for gases under pressure (Alexander 1966); pressure increases with depth, and in gas-filled swim bladders, the pressure $p_x$ in the volume $V_x$ follows the relationship $p_1V_1 = p_2V_2$. This means that a neutral buoyancy swim bladder volume of e.g. $V_1 = 250$ ml near the surface at a pressure $p_1 = 1.0$ atm will, after a dive to 10 metres in seawater ($p_2 = 2$ atm), halve its volume to $V_2 = 125$ ml, while its lifting power will be reduced to about 128 g. At 20 m and $p_2 = 3$ atm, $V_2 = 94$ ml and so on. Upward swimming leads to expansion according to the same law and a return to full volume at the surface. However, if the volume of the swim bladder is filled to neutral buoyancy volume at a depth of 50 m, the bladder will expand by 500% if the fish moves to the surface, which for most species will mean bladder rupture or loss of buoyancy control.

## Open or closed swim bladder

The anatomy of the swim bladder and the mechanism for regulating the volume of gas differ widely from species to species (Fänge 1953; Berenbrink et al. 2005), according to the depth layer to which they are adapted and how much vertical flexibility is needed. Species are classified on the basis of whether the swim bladder has a conjunction via the mouth cavity; physostome (Greek *physa* = bladder, *stoma* = mouth) or not, physoclist, (*kleistos*=closed).

*Physostome swim bladder*

The swim bladder in physostome species, such as the salmonids, is connected to the oesophagus via a short pneumatic duct (Fänge 1953). The swim bladder in many salmonids is

a ‘primitive’ elongate single chamber that extends into the body cavity, is loosely connected with the kidneys (Fahlén 1971), and has thin walls with three layers (Fig. 1; Fänge 1953).

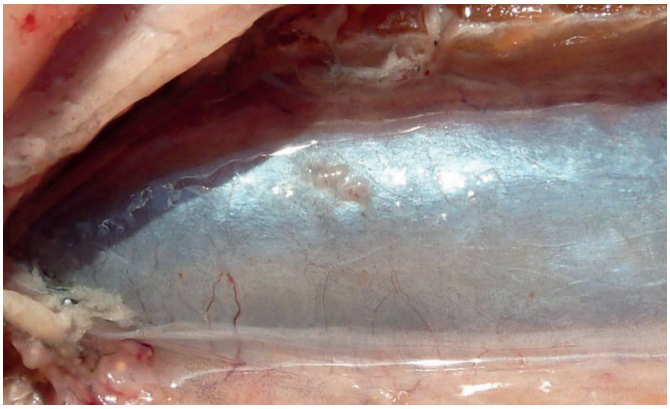

Fig. 1. Swim bladder wall in Atlantic salmon (*Salmo salar*). (Photo, Ø. Korsøen).

The method by which Atlantic salmon fill the swim bladder seems simple. Salmon are often seen swimming near the surface and either snap and swallow air during a ‘porpoising’ roll (Furevik et al. 1993) or jump out of the water. No gas secretion mechanism has been found (e.g. Fahlèn 1971). This means that this species has a very limited ability to adjust its buoyancy, and will be neutrally buoyant only close to the surface. However, other species of the salmonid family that have been denied surface access, have managed to refill empty swim bladders albeit at slow rates (e.g. Wittenberg 1958). Fahlèn (1971) reported vascular bundles, “*micro retia*”, within the swim bladder of the physostome powan (*Coregonus lavaretus)*. Similar “*micro retia*” are found in the physostome bloater, a deepwater cisco (*Coregonus hoyi*), which has a narrow pneumatic duct through which gas release seems to be restricted, as the fish bloat when brought from depth to the surface (Clemens and Stevens 2007). Knudsen and Gjelland (2004) concluded that *C. lavaretus* and *C. albula* could inflate their swim bladders without surface access, as the fish released bladder gas when ascending to the surface. The swim bladder function in physostomes is probably adapted to the depth at which the fish reside. Atlantic salmon, as a predominantly surface-dwelling fish, may lack a “*micro retia*”, or have a very low gas secretion capacity. To avoid bladder expansion during ascents,

the emptying of the swim bladder takes place via the short pneumatic duct and the buccal cavity, as the salmon appears to be able to expel gas when ascending (Ablett et al. 1989).

*Physoclist swim bladder*

The swim bladder in physoclist fish is thick-walled and dorsally connected to the rib bones. The volume may be partly divided into smaller chambers, but there is often one large chamber (Kryvi 1992). The wall consists of five layers (Fänge 1953), one of which forms glandular cells and blood vessels in the gas gland (*rete mirabile*) area where gas secretion takes place (see Fig. 2). Around the resorbent part of the bladder, one layer contains smooth muscle bundles and a capillary network adapted for gas resorption called the 'oval area'. Stretch receptors around the bladder control the gas content autonomously and continuously (Wahlquist 1985). The vagus nerve is also involved, as stimuli here increase blood flow in the capillary network in the *rete mirabile* (Schwerte et al. 1997). When the pressure inside the swim bladder increases, the oval edge rapidly opens and gas diffuses into the resorbent capillary network (Schwerte et al. 1997). The blood continues via the heart directly to the gills, and the gas may be lost to the ambient water, or utilized metabolically (Steen 1970).

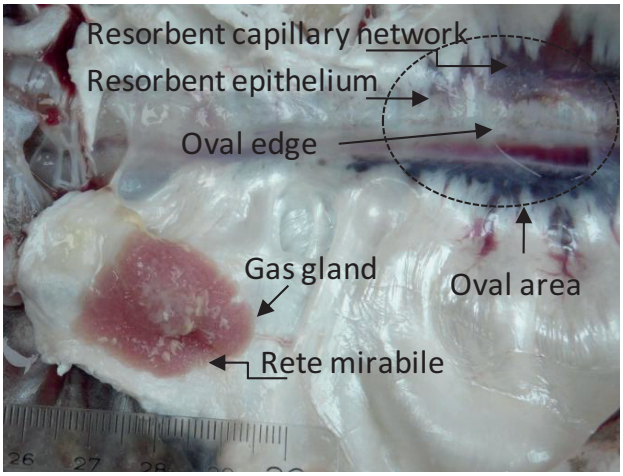


Fig. 2. Inside the swim bladder of Atlantic cod (*Gadus morhua*). (Photo, Ø. Korsøen).

### *Secretion of gas into a physoclist swim bladder*

The tension of a gas in water changes little with depth, and at the surface there is equilibrium with 0.2 atm oxygen and 0.8 atm nitrogen (Schmidt-Nielsen 1975). The same partial pressure thus exists in the blood of a fish, and the challenge for a fish with a gas-filled bladder is to fill the required volume and then maintain the pressure inside the swim bladder to reach equilibrium at the new depth.

Our current understanding of the secretion mechanism was reviewed by Steen (1970); arterial blood passing through the capillaries of the gas gland in the swim bladder epithelium is acidified by release of lactic acid and carbon dioxide in the gas gland cells. Low pH then causes a significant fall in the $O_2$-binding capacity to haemoglobins (*Root* off-shift) and $O_2$ affinity in haemoglobins (*Bohr* effect) in spite of high oxygen tension. The "*salting out*" effect in the gas gland also releases inert gases. The increase in physically dissolved $O_2$ is amplified by the tight bundle of arterial and venous capillaries running closely together against the current in the *rete mirabile*. Unloaded $O_2$ from haemoglobins in the venous capillaries diffuses back into the arterial supply to the swim bladder, and the subsequent build-up of high $O_2$ tension then secretes gas from the gas gland into the swim bladder against high pressure. The venous blood loses gas until diffusion equilibrium with the incoming arterial blood is regained, by a slow binding of oxygen to haemoglobin (*Root* on-shift).

The *Root* effect in cod is high in comparison with other teleosts. Of 49 species measured, most had *Root* effects above 40%, but with a considerable variation, from 80% in cod to 2% in a catfish (*Silurus*) (Berenbrink et al. 2005). This suggests that cod is an efficient species as far as adapting to different depths is concerned, which fits with the observation that cod are often caught at depths down to around 500 m (Godø and Michalsen 2000).

### *Gas leakage in swim bladder walls*

The gases in a swim bladder (Box 2) diffuse through the swim bladder wall depending on the pressure gradient between the bladder and the water and the permeability of the bladder wall (Blaxter 1980). Purine in the swim bladder wall reduces gas diffusion, and the content of purine is high in Atlantic cod compared to Atlantic salmon (Blaxter 1980). Leakage further

depends on the surface-to-volume ratio of the swim bladder (Strand et al. 2005), which means that a small fish will have a higher loss of gas than a larger one. To the best of our knowledge, no leakage rates have been reported for physostome fish.

***Gases in the swim bladder*** ***Box 2***

The deeper the fish live, the more oxygen there is in physoclist swim bladders, and the less nitrogen, carbon dioxide and argon (e.g. Wittenberg 1958). For example, a physoclist at 900 m depth had 75.1% $O_2$, 20.5% $N_2$, 3.1% $CO_2$ and 0.4% Arg, whereas fish near the surface had a gas content similar to air (78% $N_2$, 21% $O_2$, 0.9% Arg, 0.1% others) (reviewed by Steen 1970). In physostome species $N_2$ dominates, with the $O_2$ content ranging from 0 - 13% (Wittenberg 1958; Sundnes and Bratland, 1972).

## Coping with unbalanced buoyancy

The main disadvantage of a swim bladder as a buoyancy-control organ is that it is only neutral at a certain depth and it is easily compressed when the pressure increases and expanded when it falls, a weakness that constrains the vertical movements of the fish. The physoclist swim bladder in particular acts as a pressure balloon which is slow to fill (hours or days) and rapid to empty (minutes or hours) at great depths (Strand et al. 2005). In the meantime, the fish need to respond instantly to avoid floating to the surface or sinking too deep and losing control. Positive or negative buoyancy for short or long periods may induce stress, with the level of stress depending on whether the fish decides itself or it is forced into a situation by a predator, a fisherman (Parker et al. 2006) or a fish farmer.

### *Stress – a general overview*

The term “stress” is commonly used to describe the physiological and behavioural responses of an individual that perceives a threat to its homeostasis (Moberg 2000), i.e. the dynamic equilibrium of cellular needs and the environment needed for survival (Cannon 1929). Any threat that causes stress is known as a stressor. The purpose of stress is to enhance the body’s

tolerance of stressors, and thereby adapt to or overcome stressful situations (negative as well as positive arousal) (Selye 1976). The general stress response consists of complex neuroendocrine mechanisms that facilitate the mobilization and redirection of energy towards behavioural and physiological coping attempts (e.g. increased metabolism and ventilation frequency). As the resources of the body are limited, stress reduces the anabolic processes (e.g. growth and immune functioning) and investment activities (e.g. feeding and reproductive activity) (Barton 2002). Thus, while temporary stress can be adaptive, long-term (chronic) activation may compromise the response mechanisms and lead to pathology and maladaptive states (Barton and Iwama 1991). The degree of stress that is imposed on a fish determines its capacity to respond to secondary stressors, i.e. the responses and effects can be cumulative (Schreck 2000). To be able to respond adequately to challenges, any animal should, however, be challenged to a certain degree to improve its adaptive physical and mental performance (Galhardo and Oliveira 2009).

Physiological stress is usually categorized in terms of *primary responses*, in which neuroendocrine cascades are initiated; *secondary responses*, which are the direct effects of the primary responses, such as increased metabolism, ventilation and reduced immune function; and *tertiary responses* in which the whole animal and eventually the population are affected by changes in growth, disease resistance and reproductive capacity (Barton and Iwama 1991; Barton 2002).

Behavioural responses such as avoidance of stressors are often seen as the first line of defence. If exposure cannot be avoided, the performance of the fish needs to be modified (Schreck 1997; Galhardo and Oliveira 2009).

### *Possible stressful situations during buoyancy challenges for salmon and cod*

In the following section I will focus on how salmon and cod modify their behaviour when they become out of neutral buoyancy.

*Ascending situations*

A lifting operation or sudden ascent of physoclist cod in a standard deep net (with surface access) may lead to different stress situations, depending on the degree of pressure reduction. If the distance is within the "free vertical range" within which the fish can retain behavioural control (e.g. by downward swimming), any stress will be short-term and will diminish as gas is released from the swim bladder via the oval and the reabsorptive capillary network. It has been suggested that the "free vertical range" is less than a 50% pressure reduction, on the basis of measurements made for cod in natural habitats (Godø and Michalsen, 2000; Stensholt et al. 2002), and less than 25%, on the basis of pressure tank experiments (Blaxter and Tytler 1978; Harden Jones and Scholes 1985).

A distance slightly above the "free vertical range" will lead to an uncontrolled and highly stressful situation, where the lift force of the expanding swim bladder will accelerate the fish towards the surface. If the fish is not able to swim forcefully downward to a safe pressure area, it will end up belly up with an expanded swim bladder on the surface (barotrauma), risking an ultimately death (Gitschlag and Renaud 1994; Simolin et al. 2002).

If the pressure reduction is greater than about 70%, and thus above the "free vertical range", the swim bladder ruptures (Blaxter and Tytler 1978; Harden Jones and Scholes 1985; Midling 2006), and the gas is released rapidly inside the membrane (*peritoneum*) of the abdominal cavity. Further pressure reduction leads to the muscle tissue close to the anal opening rupturing and gas trickling out of the fish (Midling et al. 2006). The cod will be able to recover swimming control but will then have negative buoyancy. Around 80% of captured cod lifted from around 150 m depth to the surface with ruptured swim bladders survived and the swim bladder healed (Midling et al. 2006), indicating that this bursting mechanism can function as a safety valve, preventing total loss of buoyancy control (Love 1980). On the basis of these observations, the question of just how serious swim bladder rupture is for cod has been raised (Kooij et al. 2007). Nevertheless, the pain of the rupture and the time until rupture is bound to cause major stress. Several demersal physoclists such as tusk (*Brosme brosme*), do not have the same safety valve as Atlantic cod, and the expanding swim bladder squeezes the stomach and viscera out of the buccal cavity during a long and rapid ascent (pers. obs.).

Furthermore, during rapid lifting, the crowding of the school and escape behaviour of other fish may be additional stress factors, which may reduce resorption capacity, as stress leads to an ionic/osmotic disturbance (McDonough and Hemmingsen 1985; McDonald and Milligan 1997) that may limit gas transport in the blood. Finally, an inability to avoid the surface (Fernö et al. 1995) could lead to stress in cod, an effect that cannot be investigated in pressure chambers.

No previous experiments have investigated the immediate response and the recovery time after pressure reduction ascents equivalent to the free vertical range for farmed cod, as only pressure chamber experiments had been performed on captured wild cod (Tytler and Blaxter 1978; Harden Jones and Scholes 1985). Kristiansen et al. (2010) found that free-swimming farmed cod voluntarily ascended to depths equivalent to a pressure reduction of between 30-50% with an average of 41% at around 13°C at autumn. Pressure tank experiments have shown that the resorption of gas by the swim bladder occurred more rapidly (2-18 m $h^{-1}$) than secretion (0.2-1.5 m $h^{-1}$); this was positively correlated with pressure but not with temperature (Blaxter and Tytler 1978; Harden Jones and Scholes 1985).

Atlantic salmon in ascending situation are unlikely to suffer significant negative stress as they can rapidly adjust swim bladder buoyancy levels (Dempster et al. 2008).

*Descending situations*

All fish that are neutrally buoyant at the surface and are lowered in a submersible cage experience compression of the soft-walled swim bladder and negative buoyancy. In physoclist species, this activates gas secretion in the gas gland to refill the swim bladder to produce lift. In the meantime, swimming upwards or at a higher speed is necessary to create lift to avoid sinking. As physoclist species are often bottom dwellers, they can also lie and rest on the bottom, which may not be particularly stressful. In fact, such a “return” to greater depth may be no more than a brief and minor stress response for physoclist bottom-dwelling species if they are able to rest on the bottom. Neutral buoyancy is subsequently regained when the swim bladder is re-inflated, a process that in cod has been estimated to take 0.5 to 1.5 m $h^{-1}$, depending on water temperature and pressure (Harden Jones and Scholes 1985). In a farming

situation in standard nets with coned bottoms without the option of resting on the net-floor, crowded fish might be stressed, which may reduce their resorption capacity, as stress may lead to a fall in blood pH and ionic/osmotic disturbance (McDonough and Hemmingsen 1985; McDonald and Milligan 1997), limiting gas transport in the blood.

Physostome surface-dwelling species such as Atlantic salmon are unlikely to be stressed during a lowering operation, as this species often dive rapidly to great depths in nature (e.g. Westerberg 1982). If there is no access to the surface, the fish may turn to a more alert state. A long period without surface access and re-filling options will force the fish to swim continuously to avoid sinking, and the option of resting on the bottom could be limited in an oceanic surface-dwelling species; avoidance of the cage bottom has been observed in farmed salmon (Fernö et al. 1988).

Long-term high-speed swimming may gradually lead to exhaustion and load the muscles in the tail region to such a degree that some vertebrae become compressed, and such symptoms (*lordosis*) have been observed in fish forced to swim in a strong current for weeks (Divanach et al. 1997; Sfakianakis et al. 2006). Tilted swimming in Atlantic salmon was observed in small submerged cages in the sea (Fosseidengen et al. 1982) and tanks (Ablett et al. 1989). The restricted space within such small test enclosures may have inhibited fish from compensating for negative buoyancy by swimming at sufficient speed to generate hydrodynamic lift using the pectoral fins as hydrofoils (Weihs 1973; Magnuson 1978; Strand et al. 2005).

## Swimming techniques and vertical dynamic

### *Locomotion*

Although body shape and fin morphology are different between the streamlined salmon and the more anteriorly rounded cod, they both have an undulatory mode of swimming with side-to-side undulations of the body, while the tip of the snout oscillates only with moderate amplitude (Lindsey 1978). The higher content of red (aerobic) muscle fibres in Atlantic salmon than in cod raises the salmon's endurance at cruising speed (Schmidt-Nielsen 1975).

Swimming is classified into steady swimming at cruising (sustained), prolonged, and sprint (burst) activities (Webb 1978). Farmed Atlantic cod have been found to have a critical swimming speed ($U_{crit}$ used to evaluate prolonged swimming performance) that ranges from 0.7-0.9 BL $sec^{-1}$ (Lurman et al. 2009), while farmed salmon (< 800 g) have been measured at between 2-3 BL $sec^{-1}$ (Deitch et al. 2004; Wagner et al. 2004; Lijalad and Powell 2009).

The forward swimming speed creates hydrodynamic lift generated by the extended pectoral fins, which act as hydrofoils (Weihs 1973; Magnuson 1978; Strand et al. 2005). The wing-shaped body of Atlantic cod may provide lift, and swimming with a positive angle of attack (around 7-9º head up) created lift for Atlantic mackerel (Magnuson 1978) and herring (Ona 1990). Negatively buoyant herring have also been observed gliding downwards without swimming movements and then swimming to regain altitude ("sawtooth" swimming; Huse and Ona 1996), possibly saving energy (Weihs 1973). Another compensatory swimming behaviour involves remaining in a steady position, called hovering, which involves only movement of the pectoral fins. This behaviour has not been described in cod or salmon, but has been suggested as a possible behavioural adaptation for cod if they experience negative buoyancy outside their normal range (Strand et al. 2005).

### *Schooling*

Horsemackerel (*Trachurus mediterraneus ponticus*), which has no swim bladder and therefore is a continuous swimmer, were found to school in a diamond pattern, where the second row of fish swam diagonally behind and midway between fish in the first row (Zuyev and Belyayev 1970). Vortex rotation induced by the first row of fish resulted in swimming advantages around 15-20% for the second row of fish, and the optimum direction and distance to the neighbouring fish seemed dependent on swimming speed. Schooling may therefore offer hydrodynamic advantages as it may reduce drag and energy costs as the fish can stay in the slipstream of another (Weihs 1973; Sfakiotakis et al. 1999). Liao et al. (2003) also found rainbow trout (*Oncorhynchus mykiss)* to utilise vortices when swimming in strong water current (4 BL s-1) by adjusting the body as a self-correcting hydrofoil. On the other hand, schooling saithe (*Pollachius virens*), Atlantic cod and herring (all with swim bladder) did not

swim in this theoretically optimal pattern (Pitcher and Parrish 1993), likely due to their neutral buoyancy and no demand to school in order to gain lift.

In densely stocked salmon populations, a counter-clockwise ring-like structure or torus-shaped school is often observed at swimming speeds between 0.6 and 1.1 BL $sec^{-1}$ (Fernö et al. 1988; Blyth et al. 1993; Juell et al. 1995; Ang and Petrell 1998; Dempster et al. 2008). Schooling has also been suggested to correlate with increased feed intake (Fernö et al. 1988), and between meals, schooling can provide a possibility for a monotonous state which has been suggested to be a substitute for rest or sleep (Kavanau 2001).

In contrast, Atlantic cod probably have a less organised schooling structure, as they are less pelagic than salmonids, and more adapted to seek prey among the bottom vegetation (Hobson et al. 2007; Rillahan et al. 2009). Farmed cod are often found exploring the net walls and bottom, and biting holes in the net cage material (Moe et al. 2007), unlike salmon.

### *Diel vertical migration (DVM)*

Variations in light, temperature, oxygen and salinity result in vertical gradients in the sea water. As fish normally are visual predators, feeding is most efficient in daylight and shallow water (Onsrud et al. 2004). The diel or diurnal vertical migration during a 24-hour period is typically an upward movement before sunset and a downward movement before sunrise. This is found among a vast amount of organisms in the sea such as plankton (Saito and Hattori 1997), crustaceans (Fernández de Puelles et al. 1996) and mesopelagic and pelagic fish (e.g. Stensholt et al. 2002). A trade-off between optimizing food intake and minimizing risk of predation often ultimately explains these diel vertical migrations (e.g. Aksnes et al. 1990).

Wild salmonids are also found to have this vertical migration (e.g. Smith 1982), which allows them to feed in the twilight around sunset and sunrise, when typical prey species like crustaceans and euphasiids (Jacobsen and Hansen 2001) are visible and not too deep. Seasonal and ontogenetic variations also exist as post-smolt of Atlantic salmon are found drifting passively in the surface current at day time (Holm et al. 2000).

Farmed Atlantic salmon without artificial light is often observed to swim deeper in the sea-cages during the days compared to at nights (e.g. Juell and Westerberg 1993, Oppedal et al. 2001; Johansson et al. 2009), indicating that this pattern may be a natural behavioural trait. When provided submerged underwater artificial light, a shift in the DVM has occurred, with shallower swimming depths at days and descent to the lamp depth during nights (Juell et al. 2003; 2004; Oppedal et al. 2007).

The vertical movements of Atlantic cod seems difficult to categorise, as a wide range of vertical migration patterns have been recorded depending on stock, temperature and depth of water, season and ontogenetic stage (e.g. Arnold and Walker 1992; Godø and Michalsen 2000; Righton et al. 2001; Neat et al. 2006; Hobson et al. 2007; Meager et al. 2009). Vertical movement of farmed cod is poorly investigated, although farmed cod tended to distribute shallower than wild cod (Meager et al. 2009). Cod have been observed to have a diurnal vertical migration in 22 m deep sea cages ruled by the time of surface feeding (Skulstad et al. unpublished data).

### *Individual variation and social interactions*

Fish have personalities, which means that individuals with different coping styles need different motivations, learning and competitive abilities to handle the day-to-day challenges in life (Fernö et al. *in press*). Some individuals are more or less bold and risk-taking than others (e.g. Magurran, 1993). Koolhaas et al. (1999) reviewed several undomesticated populations and found that proactive and reactive coping styles continued to exist after domestication. Dominance or sub-dominance can be individual coping styles for improving foraging success (e.g. Koolhaas et al. 1999).

Social interactions may alter individual behaviour as group size increases. Avoidance of chaos to reduce stress may impose a schooling structure for farmed salmonids (Christiansen et al. 1992, Juell 1995), as increasing stocking density is assumed to decrease aggression (Ellis et al. 2002). Although social hierarchies are normal in animal populations, dominance hierarchies in salmon and cod schools in commercial sea-cages have been paid little attention, probably due to the complexity of such studies. Life in a sea-cage may be simple compared to

the lifestyle of their wild counterparts, as threats from human handling are rare, food is easily available and environmental conditions often within acceptable limits. However, serious challenges may arise if the numbers of conspecifics become too high, escalating if environmental stratifications appear and the space becomes limiting (Johansson et al. 2006; Oppedal et al. 2011b).

## AIMS OF THE THESIS

To provide protection from poor surface conditions such as wave-swept surface water, the general aim of this thesis was to describe short- and long-term behavioural responses and welfare parameters of the physostome Atlantic salmon and physoclist Atlantic cod subjected to changes in their buoyancy status to different degrees and periods of time. The first specific aim was to identify and describe the swimming behaviour, growth performance and conditions at group-level of Atlantic salmon cultured in fully submerged cages at shallow level for short duration with artificial light at night to enable compensatory swimming both during the day and at night (Paper I). The second aim was to measure the same parameters in a "worst-case" situation where large Atlantic salmon were submerged to greater depth and longer time without additional light during dark winter nights (Paper II). The third aim was to describe the vertical movement of individual salmon in a school under the two described submerged conditions, to determine whether different coping abilities among individuals existed (Paper III). The fourth aim was to test a protocol for safe lifting and lowering of submersible cages with free swimming farmed Atlantic cod and on the basis of the behavioural responses to identify safe acclimation times before the next vertical step at warmer (16°C) and colder (5°C) sea temperatures (Paper IV).

## ABSTRACTS OF PAPERS

### *Paper I*

Submergence of Atlantic salmon (*Salmo salar*) in sea-cages: a potential short-term solution to poor surface conditions.

Tim Dempster, Øyvind J. Korsøen, Ole Folkedal, Jon-Erik Juell, Frode Oppedal.

Submergence of Atlantic salmon (*Salmo salar* L.) in commercial scale sea-cages (1600–2000 $m^3$) affected their behaviour, but did not alter growth rates, food conversion ratios, appetite, condition factor or fin condition in comparison with control cages held under similar environmental conditions. Four sea-cages each held 3300–4200 Atlantic salmon of 0.45 kg; two cages acted as controls, while two were submerged for 22 days with the roof held at 3 m depth. Salmon in the control cages mainly swam at similar depths to those maintained by submerged fish, resulting in both treatments experiencing similar temperature, light, salinity and dissolved oxygen levels. Submerged fish swam an average of 1.6 times faster (0.88 BL $s^{-1}$) and in more structured schools than control fish (0.55 BL $s^{-1}$). Specific growth rates (1.1–1.3% $day^{-1}$), appetite (0.8–1.2% body weight $day^{-1}$) and condition factor (1.10–1.15) were high, while food conversion ratios (0.6–1.0) and the incidence of fin damage were low in all cages, with no significant differences between the control and submerged treatments. Our results highlight the potential for submergence of salmon in sea-cages for short periods to avoid negative surface events.

### *Paper II*

Long-term culture of Atlantic salmon (*Salmo salar* L.) in submerged cages during winter affects behaviour, growth and condition.

Øyvind J. Korsøen, Tim Dempster, Per Gunnar Fjelldal, Frode Oppedal, Tore S. Kristiansen.

In the search for alternative farming methods, we investigated whether large salmon submerged below 10 m in winter conditions behaved normally and performed as well as

control fish held in standard surface cages. On average, 2345 salmon of ~3.5 kg were kept in each of six 2000 $m^3$ sea-cages for 6 weeks; three of which were submerged to 10–24 m depth and three acted as surface controls (0–14 m). Behaviour during both day and night was studied with echo-sounders, and underwater video cameras fitted with infra-red lamps. A sub-sample of fish from each cage was weighed, measured and assessed for fin and snout condition prior to and after the experimental period. In addition, the vertebral column of 50 fish from the control and submerged treatments were dissected and X-rayed to assess vertebral deformities. The submerged salmon seemed unable to re-fill any gas into the swim bladder, as a linear decrease in echo reflection to <5% of presubmergence levels after 22 days of submergence indicated loss of almost all gas from the physostomous swim bladders and negatively buoyant fish. Around day 22, submerged salmon swam at night time with a distinct 'tail-down, head-up' tilt (26°) compared to the horizontal swimming position of control fish (−3°). Average swimming speed (body length per second) of submerged salmon were 1.3–1.4 times faster (day: 0.77±0.02; night: 0.46±0.02, (mean±SE)) than control fish (day: 0.54±0.01; night: 0.37±0.02) both during day and night. Almost no mortality was seen, and the submerged salmon maintained similar diurnal vertical migrations as the surface fish, indicating that deep submergence did not exhaust the fish. However, submerged fish fed less efficiently, resulting in lower growth and reduced feed utilization. Fins and snouts of the submerged fish had small, but significantly more erosion than the control fish. Vertebrae in the tail region were significantly compressed in the submerged fish compared to control fish. This could be an early symptom of development of vertebral deformities. The results suggest that continuous submergence below 10 m for longer than 2 weeks reduces the welfare and performance of Atlantic salmon.

### *Paper III*

Individual variation in growth and vertical swimming behaviour in Atlantic salmon (*Salmo salar* L.) subjected to submergence in sea-cages.

Øyvind J. Korsøen, Tim Dempster, Frode Oppedal, Tore Kristiansen.

Large individual variation exists in the coping repertoire of Atlantic salmon (*Salmo salar*) in response to environmental changes in sea-cages. We compared the growth and behaviour of individual salmon within and between submerged (no surface access) and standard cages (with surface access), using high resolution data storage tags (DSTs). Two different commercial-scale experiments were conducted; one with 0.5 kg salmon (n = 2300), submerged below 4 m for 22 days with 24 h continuous light, and the other with 4 kg salmon (n = 4800) submerged below 10 m for 42 days with natural light. Shallow, short and illuminated submergence resulted in a diurnal pattern similar to control fish, although with slightly less variation among individual swimming depths at night and more during the day. Individuals with highest growth rates tended to swim deeper at night at end of the trial. Salmon exposed to longer, deeper and dark submergence conditions displayed more irregular diurnal swimming patterns. Large variations in coping strategies were evident, likely as a result of differing levels of negative buoyancy among fish, possibly caused by deflated swim bladders. Submerged individuals with high growth rates swam either with a large amplitude diurnal cycle, or deeper during the day compared to fish with lower growth rates. In addition, submerged fish with high growth rates displayed more vertical activity during feeding. Short-term, illuminated and shallow submergence resulted in little behavioural deviation compared to the control fish, indicating that it did not compromise welfare. However, the more challenging deep, dark and long-term submergence with large differences in individual coping styles and shifts in diurnal swimming patterns compared to control fish, suggest compromised welfare for individual fish that coped poorly.

### *Paper IV*

Behavioural responses to pressure changes in cultured Atlantic cod (*Gadus morhua*): Defining practical limits for submerging and lifting sea-cages.

Øyvind J. Korsøen, Tim Dempster, Jan Erik Fosseidengen, Anders Fernö, Einar Heegaard Tore S. Kristiansen

Farmed Atlantic cod (*Gadus morhua*) are occasionally exposed to buoyancy changes in sea-cages, through lifting or lowering of cage nets. Physiological processes regulate the level of

gas in the closed swim bladders of cod and thus the ability of cod to control their buoyancy. Rapid net lifting may cause positive buoyancy, leading to barotrauma, while net lowering may lead to negative buoyancy and alter cod behaviours. We tested how groups of farmed cod responded immediately after lifting events from 5 different start depths equivalent to 40% pressure reductions, and how long they took to return to pre-lifting pressure levels. In addition, we tested immediate responses and recovery times to cage lowering events equivalent to 100–300% pressure increases. Trials were conducted with 100 cod of 1.1–1.7 kg in a 63 m3 sea-cage at the lower (5 °C) and upper (16 °C) water temperature limits experienced during culture. Swimming behaviours were measured at fixed intervals before and after cage lifting or lowering, and a feeding test was used to assess appetite. In general, lifting events increased swimming speeds 1.5–4 times and tail beats 2–3 times and fish swam with an average −14° head-down angle, indicating positive buoyancy. The depth before lifting affected the immediate response as the fish became more active after lifting events from shallow compared to deeper depths. Appetite levels decreased for about 2 h after cage lifting, independent of temperature or start depth. The overall recovery time of 8 h after lifting did not depend upon start depth or temperature. Lowering events appeared to cause negative buoyancy. Swimming speeds (1.3–2.3 times) and tail beat frequencies (1.4–2.3 times) increased immediately after cage lowering, and cod swam with an average 30° head-up swimming angle. Neither pressure level nor temperature affected this immediate response. Time to recover to neutral buoyancy for 300% pressure increases took 42–90 h, but only 18–34 h for 100% pressure increases. We conclude that a 40% pressure reduction is an upper limit for lifts of healthy farmed cod. Secondary lifts should not be done until at least 10 h after the first lift. Cage lowering should be done slowly to avoid potentially stressful crowding of negatively buoyant fish on the cage bottom, especially at low temperatures.

# DISCUSSION

This thesis demonstrates how short-term shallow submergence of Atlantic salmon in large sea-cages did not lead to negative consequences if a reference light was given to enable compensatory swimming both during the day and at night (Papers I and III). Deeper long-term submergence in winter with long dark nights with no reference light had a number of physiologically and behaviourally negative consequences for the fish (Papers II and III).

Atlantic cod managed to regain neutral buoyancy at all depths at which they were forced to swim (Paper IV). Their high swimming activity indicated that a 40% pressure reduction is an upper limit for safe lifting. Rapid lifting events were more challenging when the cod ascended to the surface. Independent of water temperature (5 and 16°C), cod needed around 8-10 h to recover their neutral buoyancy after being raised from five depths ranging from 30 to 8 m.

## Research approach and experimental conditions

I aimed to study the biological criteria for submergence of farmed Atlantic salmon and cod under realistic farming conditions, where the fish were allowed to swim freely to express their behavioural responses to pressure change.

In all experiments, the environmental conditions in the submerged and control surface cages were similar. No sub-optimal conditions such as algal blooms, extreme temperatures or heavy sea-lice infestations were observed. The results should therefore not be affected by environmental differences, but mainly be due to changes in pressure and denial of access to the surface.

### *Atlantic salmon*

In the first experiment (Paper I), a stocking density of 1.5 kg $m^{-3}$ (mean fish weight 0.5 kg) was chosen. This density is close to a realistic stocking density for post-smolt farming (Juell et al. 2003; Juell and Fosseidengen 2004) compared to the density in a pilot study (Dempster et al. (2008); stocking density 0.7 kg $m^{-3}$). Even higher densities were used in the second

experiment (Paper II), with a stocking density of 5.5 kg $m^{-3}$ (mean weight 3.5 kg). Submergence of salmon heavier than 2.5 kg (Ablett et al. 1989) has not previously been investigated.

The sea-cages in the Cage Environment Laboratory at the Institute of Marine Research field station at Solheim are 12 × 12 m across and 14 m in depth (~1800 $m^3$). In net cages of such a size the salmon can swim freely and at high speed, while at the same time we can measure parameters such as biomass and feed intake with reasonable accuracy. Previous attempts to determine the effects of submergence on salmonids have been made in tanks and small cages. In these experiments, rapid onset of tilted swimming, loss of buoyancy control and, in some cases, exhaustion and mortality, have been observed (Fosseidengen et al. 1982; Ablett et al. 1989). In contrast, the few pilot tests that have been performed in large sea-cages (Osland et al. 2001; Dempster et al. 2008) have indicated that salmon can cope with submergence, as they fed actively and grew normally, although at a slower rate than the control cages held at the surface. For 'high-performance' swimmers such as Atlantic salmon, it was therefore regarded crucial to provide sufficient space to enable them to express their behavioural repertoire to adapt to changes in buoyancy.

The experimental conditions for Paper I were designed to deny access to the surface and to provide only a slight increase in pressure. The salmon were submerged below 4 m by lowering a roof, similar to the procedure of the pilot test (Dempster et al. 2008). The duration of 22 days was chosen to evaluate a slightly more severe challenge than in the pilot study, and submerged lamps at a depth of 7 m were provided to enable the salmon to swim at speeds sufficient to enable schooling to avoid tilted swimming at night (as for negatively buoyant scombrids (Zuyev and Belyayev 1970)). Dispersed swimming in darkness is often observed in farmed salmon under natural light conditions (Juell and Westerberg 1993; Oppedal et al. 2001; Johansson et al. 2009), whereas salmon will school when light is provided at night (Juell et al. 2003; Oppedal et al. 2007). The light enables the fish to perceive neighbouring fish in the school as well as the walls of the sea-cage, conditions that are essential to allow compensatory swimming speeds and to avoid collisions. This treatment is also a standard method utilised to prolong the time before sexual maturation in farmed salmon (e.g. Oppedal et al. 1997).

As the results from this experiment were promising with regard to growth and swimming behaviour, a new "worst-case" experiment was designed (Paper II) to measure the response of larger salmon (3.5 kg) submerged more deeply (10 m) and for longer (42 days) in winter without any additional light. The depth of 10 m was chosen to be relevant to commercial conditions, as it would force the fish away from possible unfavourable surface conditions such as heavy waves and strong currents (Ryan 2004), sea lice infestations (Johanessen 1978) and fluctuating environmental conditions (Johansson et al. 2007; Oppedal et al. 2011b). The choice of no artificial light was based on the knowledge that light treatment cannot be used continuously all year round, as continuous light must be given only in periods (e.g. Oppedal et al. 1997).

*Measured parameters*

Performance parameters were measured as weight gain and feed utilisation during the experimental period. In Paper I (Sub22), weight gain was calculated on a sample of 150 randomly selected individual fish and 50 PIT-tagged fish (total 200) from each cage. In the experiment for Paper II (Sub42), 100 fish were PIT-tagged and weighed in each cage prior to and 7 days after the submergence, and all the fish were also killed and weighed in a processing plant 14 days after the end of the submergence period. Feed rations were adjusted daily so as to keep the fish satiated. In Sub22, a re-circulating feeding system was used, in which uneaten pellets were collected, dried and weighed. This system provided high-accuracy feed utilisation evaluation. Due to technical limitations, a simpler feeding control method was used in Sub42, where the same operator continuously observed feeding activity and uneaten pellets in each cage via a remotely controlled underwater camera. Feeding rations were estimated according to Talbot et al. (1999), with two stepwise feeding rates to avoid waste of feed. Nevertheless, without a recollection system for uneaten pellets, the calculations of feed utilisation were thus liable to a somewhat larger range of error in Sub42 than in Sub22.

A range of individual behaviours were used as indicators of coping ability and welfare, in combination with group performance and behaviour. Behaviour can be used as an indicator of welfare status (Schreck 1990). Group behaviour was monitored by an echo-integration system with transducers positioned below the mid-point of each cage (Papers I and II). Several

parameters were calculated to describe the vertical position of the school and cage-volume utilisation, e.g. maximum observed fish density ($OFD_{max}$) over all depths at a given hour, max depth at the depth of the $OFD_{max}$ and preference index (PI, use of available space). The total target strength (from the echo intensity) provided in addition an estimate of swim bladder gas content, although tilted swimming reduces the total echo strength (Nakken and Olsen 1977; Juell and Fosseidengen 1995), however insignificantly in this experiment. From underwater cameras, swimming speed was calculated four times on selected days prior to, during and after the submergence period in Sub22. A total of 7200 instantaneous swimming speeds were measured, and the residuals of these measurements were used to indicate the degree of schooling (Paper I). A similar setup was utilised in Sub42, where infrared lamps connected to the underwater cameras allowed night observations of swimming speed and tilt angle to be made (Paper II), due to clear water and high visibility at this time of year.

Jumping and rolling behaviour at the surface were monitored throughout the experimental period in Sub22 in the control cages, while the submergence treatment cages were only observed on the three days before and after submergence. The total numbers of jumps and rolls in each cage during four 5-min periods were counted. Surface activity was also recorded with a video camera 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 120 min after the two submerged cages were re-surfaced (Paper I).

Pre-programmed data storage tags (DST) that measured swimming depth and body temperature at intervals of 1-30 minutes depending on the time of day were inserted into the body cavity of 51 fish in Sub22 and in 28 fish in Sub42. In Sub22 and Sub42, 42 and 26 tags respectively were used for individual analyses of diurnal vertical swimming movement (DVM) throughout the experimental periods (Paper III).

Sea lice (*Lepeophtheirus salmonis*) infestations were recorded and the fin condition of all tagged fish in each cage was assessed at the beginning and end of the experimental periods by identifying the condition of the dorsal, caudal, anal, pectoral and pelvic fins based on Hoyle et al. (2007) (Papers I and II). The snout condition of the tagged fish in Sub42 was scored as 1 for any sign of skin wear or damage and 0 if no damage was evident. At harvest, 50 randomly selected PIT-tagged salmon in Sub42 from each treatment were dissected for their vertebral

columns, which were x-rayed to reveal any deformities (Paper II). Snout and vertebrae were not judged in Sub22, due to the lack of observed collisions or tilted swimming at night.

### *Atlantic cod*

Short- and long-term behavioural responses to rapid descents and ascents of Atlantic cod inside a submersible sea-cage were studied in Paper IV. A group of 100 Atlantic cod weighing 1.1-1.7 kg was kept in a 5 × 5 × 2.5 m deep cage designed to keep the fish within a narrow pressure range. The stocking density was 1.8 kg $m^{-3}$, which is towards the lower end of the range of commercial rearing conditions. The group of cod had sufficient room to swim freely or rest on the flat net bottom. Previous studies of swim bladder secretion and resorption rates have been carried out in small pressure chambers (e.g. 37 litre, 76 cm length; Harden Jones and Scoles 1985). Such a very small volume may well induce unknown stress factors that bias the results, as will presumably the presence of only few individuals in the tanks.

Kristiansen et al. (2010) found that free-swimming conditioned farmed cod responding to a feeding signal voluntarily ascended to depths equivalent to a pressure reduction between 30-50% with an average of 41% at around 13°C in the autumn, and suggested that 40% pressure reduction giving 70% swim bladder expansion (according to Boyle's Law, 100 ml gas at 10 m (2 atm) will expand to 170 ml at 2 m (1.2 atm)). This may set a limit to how much a sea-cage with farmed cod can be rapidly lifted in a single operation, giving an indication of the "welfare limit" of the individual cod. A 40% pressure reduction was therefore used as the limit for the experiment (Paper IV). In a farming situation, crowded fish may be stressed during rapid lifting events, which may reduce their resorption capacity, as stress may lower blood pH and produce an ionic/osmotic disturbance (McDonough and Hemmingsen 1985; McDonald and Milligan 1997) that would limit gas transport in the blood. A point of uncertainty was whether a 40% pressure reduction could leave an inadequate safety margin for the possible short-term stress and exhaustion caused by the increased swimming activity needed to compensate for the positive buoyancy after pressure reduction.

Possible surface avoidance by cod, as has been observed in Atlantic salmon (Fernö et al. 1995), cannot be investigated in pressure chambers, and I wished to test whether fish

responded differently when they were raised from different start depths. The shallowest step from 8-0 m was predicted to be more challenging than the 30-14 m step.

Furthermore, the long-term recovery time to neutral buoyancy after ascents for farmed cod in free swimming conditions had not previously been studied, and only results of resorption rates for wild captured cod in pressure chambers existed (Tytler and Blaxter, 1978; Harden Jones and Scholes, 1985). Strand et al. (2005) developed a model of buoyancy control in Atlantic cod in which the lift obtained from swimming was added to earlier models based on pressure chamber results by Harden Jones and Scoles (1985). The model predicts the time needed for cod to establish neutral buoyancy after a pressure change, and the results in the present experiment were compared with this model.

The short- and long-term swimming behaviour of submerged cod at three pressure increase steps was investigated. These steps corresponded to pressure increases of 100% (0 to 10 m submergence), 200% (0 to 20 m) or 300% (0 to 30 m). Gas secretion rates into the swim bladder depend on temperature and pressure, and in a pressure tank wild cod reached neutral buoyancy at a rate of approximately 0.5 m $h^{-1}$ at low temperature (0-6°C) and low pressure (1-3 atm), and at 1.5 m $h^{-1}$ at higher temperature (13-17°C) and higher pressure (4-6 atm) (Harden Jones and Scholes, 1985). Here too, the results were compared with the predicted values from the model of Strand et al. (2005).

To test the effect of temperature on gas exchange rates in the swim bladder of free swimming farmed cod, the experiments were performed during both warmer (16°C, September) and colder (5°C, March/April) times of year. The times were chosen on the basis of historical temperature logs at the sea-cage facilities at Austevoll Research Station.

*Behavioural measurements*

Swimming speeds, swimming tail beats and tilt angle were measured from video observations made by a remote-controlled underwater camera positioned in the centre of the cage. In each observational period, 30 measurements of all three behavioural variables were made 10 min before the lifting or submergence steps, and 0.5, 1, 1.5, 2, 4, 8 and 24 hours after lifting or submerging. Recordings were also made 28, 32, 48, 52, 56 and 72 h after the submergence

steps. A total of 10560 instantaneous swimming speeds, tail beats and tilt angles were measured. The appetite response of the group of cod was estimated using video observations during each period of observations. The number of uneaten pellets left, out of a total of 20 fed to the 100 fish through the submerged feeding pipe, was determined.

After the submergence steps, the proportion of cod resting on the net-bottom was used in addition to the tail beat measurements as an indicator that the fish were refilling their swim bladders and returning to neutral buoyancy. To strengthen the results, an echo integration system was used to measure the echo intensity from the fish in the last round of experiments, as an unexpected number of cod remained resting on the net-bottom.

### Number of replicates

Replications of trials are important in aquaculture studies, as group behaviour may differ between identical rearing units (Ruohonen 1998). The position of the cage relative to water currents and other cages may create dissimilar conditions (Johansson et al. 2006; 2007). Other factors such as boat traffic and human disturbance may also affect the fish. The numbers of individuals with different coping styles and sensitivity to stress may lead to 'bad cage culture' or 'good cage culture' (Fernö et al. 1988; Juell 1995).

In the first experiment with salmon (Paper I), two cages were submerged and two used as control replicates, as submergence below 4 m was predicted as a mild stressor with relatively similar water conditions in the control cages and the submerged cages in terms of temperature, salinity, oxygen and current. In Paper II, three replicated experimental units were used as the doubling of water pressure would halve the swim bladder volume and therefore lifting power, and there were potentially larger differences between the water conditions in the control cages and the submerged cages.

In the experiment with the physoclist cod (Paper IV), only one cage was used, but repeated experiments were run in both low and high temperature conditions. As gas secretion and resorption in the swim bladder are physiological processes, I have no *a priori* information to suggest that one step should have been affected by the preceding step in each experiment, and no such trend was seen in the data. Each lifting or lowering step in an experiment was

therefore regarded as a replicate for subsequent analyses. Before a lifting or lowering step was performed, we ensured that the fish had acclimated to the new depth, as evaluated by the behavioural indicators before the next lowering or lifting step was undertaken.

To avoid error from subjective selection of individuals, the behavioural parameters were manually measured from 30 individual fish randomly chosen from the video images and films.

## Discussion of results – Atlantic salmon

### *Tolerance of negative buoyancy in Atlantic salmon*

*Swim bladder gas leakage and possible secretion*

Based on the hydro acoustic measurements, the swim bladder gas volume decreased in an almost linear fashion and in both experiments the bladder appeared to be empty after 22 days of submergence (Papers I and II), indicating that the leakage rate is independent of depth and fish size in Atlantic salmon within the range of 4 - 10 m depth and 0.5 and 4 kg. Gas diffusion rates from the swim bladder of Atlantic salmon have not previously been reported. Blaxter (1980) claimed that there is a general leakage from the swim bladder that depends on the pressure gradient between the swim bladder and the surrounding tissues that are at a lower partial pressure, the permeability of the swim bladder wall and the swim bladder's surface area. The relative gas-loss by diffusion may also depend on the surface-to-volume ratio of the swim bladder, and this ratio changes with fish size (Strand et al. 2005) with smaller fish leaking more gas than larger fish at the same depth. These effects could not be documented in my experiments where small 0.5 kg fish at shallow submergence were compared to 4 kg salmon at deeper submergence. An alternative explanation of the observed similar leakage rate can be that the leakage rate increase with depth and decrease with size, and in our case these effects cancelled each other out. Alternatively, the range of pressure-difference may have been too small, and descents to larger depths for different sized fish might have given an effect.

There was no indication of secretion of gas into empty swim bladders as observed in rainbow trout (Wittenberg 1958; Fahlén 1971) and Arctic charr (Sundnes and Bratland 1971) in the present experiments. Morphological differences among physostome species exist (Berenbrink et al. 2005), and deepwater-adapted physostomes have a *micro retia* in the swim bladder wall that produces gas (Clemens and Stevens 2007), although the mechanism involved is not fully understood. Atlantic salmon, regarded as a surface-dwelling species (Holm et al. 1982; 2000) presumably lack efficient *micro retia*, but we cannot exclude the possibility that there is long-term slow gas secretion, as gas-filled swim bladders have been found in Atlantic salmon with malformed and blocked pneumatic ducts (Poppe et al. 1997).

*Growth and feed utilisation*

In the 0.5 kg salmon submerged for 22 days, growth at the group level was similar to the control fish but with slightly lower feed utilisation (Paper I). In contrast, the large salmon (4 kg) submerged under different conditions in Sub42, exhibited reduced appetite, growth and feed utilisation (Paper II). Due to high variation between the low numbers of cages, the growth rates between the individual tagged fish broaden the picture of the growth performance. In both experiments, substantial variation in growth rates between individual fish in both the control and the submerged cage were found (Paper III). In Sub22, both groups had relatively high mean growth rates compared to Austreng et al. (1987), but the submerged DST fish had significantly lower mean growth rates than the control fish (mean SGR 1.05 vs.1.25% BW $day^{-1}$).

In the Sub42 experiment, the control fish grew faster (mean SGR 0.45% BW $day^{-1}$) than the estimated 0.36 % BW $day^{-1}$ (Austreng et al. 1987), and significantly faster than the submerged fish (mean SGR 0.20 % BW $day^{-1}$). Excluding one outlier, all the control fish grew above 0.36 % BW $day^{-1}$, while only one of the submerged fish grew above this rate. Three fish in the submerged cage had negative or no growth.

*Swimming behaviour and possible stress imposed by submergence*

Despite no immediate gas loss from the swim bladder, salmon in both experiments modified their swimming behaviour on the first day after submergence, maintaining a swimming speed that was 1.3-1.6 times higher than the control fish, and swam in more structured schools. This change persisted throughout the submergence period, indicating a lack of habituation to the increased pressure conditions in the submerged cages.

Why did the onset of faster swimming occur almost immediately following submergence? The rapid lowering of the sea-cage did not seem to induce a stressful situation, as no changes in depth indicative of immediate diving were observed during the 20-min roof-lowering operation in either experiment. Deep exploratory dives were made by salmon in Sub42 during the hour after submergence, whereas no such behaviour was seen in Sub22 (Paper III). Wild salmonids are known to dive frequently in coastal waters at certain times (Døving et al. 1985;

Walker et al. 2000; Tanaka et al. 2001; Reddin et al. 2004; Skilbrei et al. 2009). In this case, diving may have been attempts to avoid the disturbance caused by the descending roof positioned at 10 m, as avoidance is a common behavioural attempt to avoid a stressful incident (Schreck 1997).

It is still not clear why the onset of faster swimming occurred almost instantly in Sub22, since the net roof was placed at a depth of only 4 m, and thus did not cause a significant pressure increase and the fish should have approximately neutral buoyancy near the roof. The denial of surface contact, probably stressed the salmon and made the salmon more alert, as also suggested by the greater range of swimming depths during the day among the salmon in Sub22, and the shift of diurnal vertical pattern in the salmon in Sub42 (Paper III). Based on the frequent jumping behaviour after removal of the net roof (see below), surface access seems to be a strong behavioural need in Atlantic salmon.

The denial of access to the surface may inhibit a need for daily rest. The vertical diurnal swimming depth of Atlantic salmon that remained at shallower depths at night (Juell and Westerberg 1993; Oppedal et al. 2001, Juell and Fosseidengen 2004; Johansson et al. 2006), with more dispersed and slower swimming speeds than during the day (Juell 1995; Bégout et al. 1999) may suggest that there is a need to rest at night. Ninety-two percent of the DST-tagged control fish swam at very constant depths at night. Observations with an infrared camera indicated that salmon in the control cages swam in a dispersed shoal, with several fish "hanging" in the water with their head pointing slightly downwards (angle: -2 to -3°, Fig. 3A) in a way not previously observed in farmed salmon. Therefore, the more structured daytime schooling (more constant swimming speed and lower packing density) of the submerged fish that was observed in both experiments could suggest that this behaviour was a substitute for a daily rest, as schooling may be a substitute for rest or sleep (Kavanau 2001). The artificial light enabled the fish in Sub22 to swim at greater distances ('diamond' pattern), probably in the slipstream of neighbouring fish, a pattern that may have offered hydrodynamic advantages while maintaining neutral buoyancy at a low energy cost (Zuyev and Belyayev 1970; Weihs 1973; Sfakiotakis et al. 1999). The control fish swam more slowly and closer to each other (higher packing density, Papers I and II), but also at more randomly distributed individual swimming depths (Paper III), indicating a relaxed state as the swim bladder provided lift.

The more or less total darkness at night for the salmon in Sub42 imposed tilted swimming from around day 10 (Paper II and Fig. 3B). An additional negative factor for the submerged fish in Sub42 was the possible downward water current induced by the tilted swimming pattern at night, observed as small particles sinking more rapidly than in the control cages (Fig. 3). This current may have made it even more difficult to remain at a certain depth, and such observations demonstrate the importance of doing experiments under relevant farming conditions. The tilted swimming may have placed greater loads on the spine of the submerged salmon than the control fish, leading to early signs of the development of vertebral deformation. Swimming head-up tail-down may result in a gradual upward movement followed by a downward movement with weaker tail beats, as has also been described in Chinese sturgeon (Watanabe 2008). This may be shown by the wider range of individual vertical swimming depths at night (Paper II).

Tilted swimming was not observed in the 0.5 kg submerged salmon in Sub22 at night. Ablett et al. (1989) found that Atlantic salmon smaller than 1 kg swam less tilted than larger salmon of ~ 2 kg, and claimed that smaller salmon coped better with negative buoyancy than larger salmon, although the small trial unit may have limited compensatory swimming behaviour for the larger fish. As my studies were done under different light and pressure conditions, I cannot evaluate this finding.

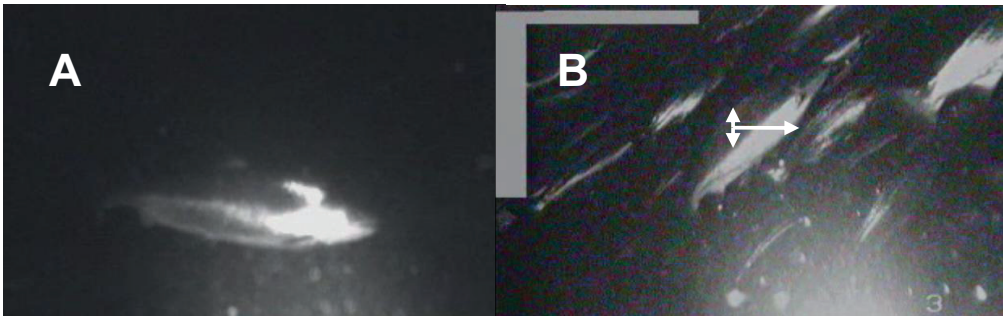


Fig. 3. Swimming behaviour at night 40 in Paper II in A) control fish slowly gliding in almost horizontal position, and B) submerged fish. White arrows indicate that the thrust generated by the tilted body has a vertical component to avoid sinking. Particles in the water were sinking rapidly in B, suggesting a vertical water-current that was probably imposed by the downward tail-beat vortexes.

The schooling structure for the submerged salmon in Paper II changed during the experimental period, and an example is shown in Fig. 4 near the end of the experimental period, when the salmon in the submerged cage were clearly negatively buoyant. This was particularly evident during feeding; while the control school had a counter-clockwise torus shape throughout the experimental period (Fig. 4A), the submerged school became a taller torus with a smaller diameter (Fig. 4C). This is supported by the higher use of vertical space (lower preference index) in the submerged school during the day and the wider range of individual swimming depths. During feeding, the control fish dynamically changed depth and often spiralled, accelerating to the feed pellets in the centre (Fig. 4B) as seen in batch feeding salmon (Ang and Petrell 1998), whereas the submerged fish seemed to move within a more limited vertical range (Fig. 4D).

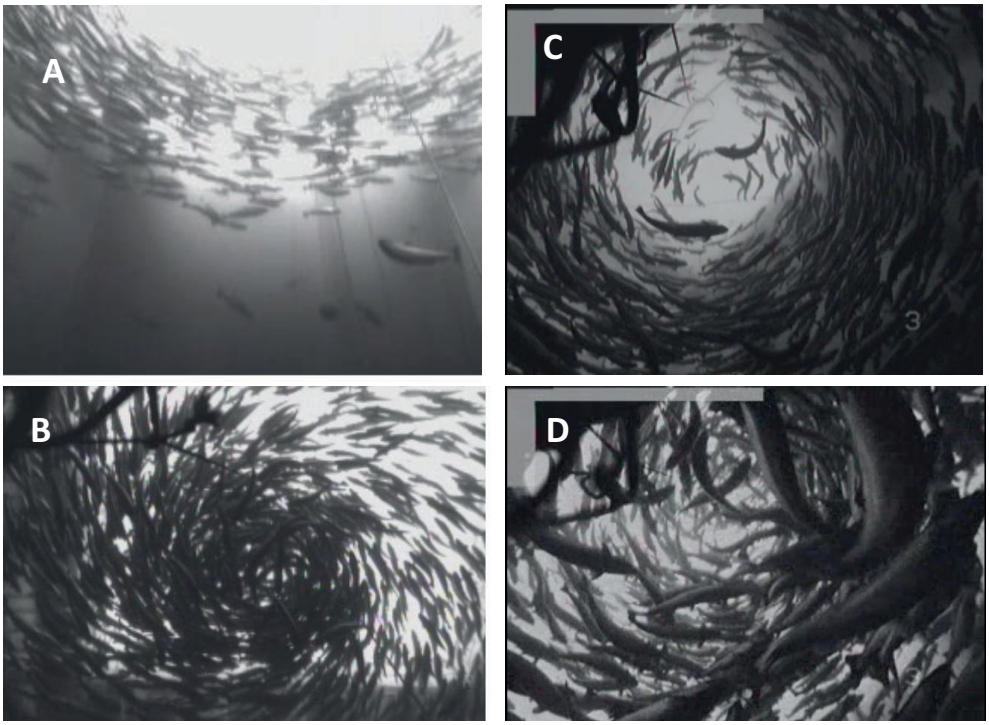


Fig. 4. Example of schooling structure of farmed Atlantic salmon (mean weight 4 kg) 36 days after the start of the experiment. A) control cage before feeding, B) control cage during feeding, C) submerged cage before feeding and D) submerged cage during feeding (Korsøen unpublished data).

*Diel vertical migration (DVM) pattern of individual fish*

Individual DVM patterns were observed in both the submerged and control fish (Paper III). The behaviour in standard surface cages with under-water light has earlier been reported only at the group level (Juell et al. 2003; Juell et al. 2004; Oppedal et al. 2007). The study of individual fish demonstrated more clearly than the group study that the fish ascend to the surface around sunset and sunrise (Sub22, Paper III), probably because of the changing light intensity and the fish search towards preferred light conditions. This could be when the salmon refill air, which is supported by the slightly greater surface activity of salmon at sunrise and sunset (Furevik et al. 1993; Blyth et al. 1993). At night they dived below the light (7-14 m), and stayed around 2-5 m during the day. Without artificial light (Sub42, Paper III), the control individuals exhibited a broadly similar diurnal pattern in swimming depth throughout the submerged experimental treatment. They ascended around sunset and swam at 2-9 m at night compared to 5-10 m at day time, and 7 out of the 13 tagged fish had large diurnal amplitudes.

Submergence in Sub22 did not cause a behavioural shift from normal swimming patterns; regardless of the submerged or control treatment, all the tagged fish exhibited a similar diurnal swimming pattern throughout the experimental period, with shallowest swimming depths during the day and deeper swimming depths around and below the artificial lights during the short nights. However, the individual swimming depth for the submerged fish varied more in daytime during the submerged period.

In contrast, the longer, deeper submergence under natural light conditions in mid-winter (Sub 42) resulted in a diurnal swimming pattern that deviated from normal for the majority of submerged individuals. The swimming pattern seemed disturbed the first 10 days of submergence, whereas the salmon adjusted to a more normal diurnal pattern the next 10 days. From day 18 and onwards, a wider range of swimming depths occurred during nights, and more fish exhibited reversed or irregular DVM during the last 20 days. The two last days of submergence, 8 of 13 tagged fish exhibited an reversed DVM with deep nights (~16 m) and shallow days (~12 m), or swam at similar depths during both night and day. 5 of the 13 tagged salmon exhibited a more normal pattern, with shallow nights (~11 m) and deep days (~16 m).

*Fish at different depths have different growth rates*

In both experiments, the fish with the highest growth rates swam deeper in the submerged cages. This pattern emerged from around 10 days post-submergence in Sub42 during the day, and at the end of Sub22, but only at night. As the swim bladders of all individuals were probably empty after 22 days in both experiments, an assumption based on the group-level echo-sounder measurements (Papers I and II), individual variations in swim bladder fullness are presumably not the main cause of this pattern.

High stocking densities of salmon in sea-cages have been reported to result in severe negative welfare implications, such as fin erosion, stress, reduced appetite and mortality (Ellis et al. 2002; Turnbull et al. 2005; Oppedal et al. 2011b). Environmental stress can also increase aggressive behaviour (Beitinger 1990). In spite of the relatively low densities in this study, the gradually declining buoyancy may have stressed the submerged fish, which may in turn have induced the development of a dominance hierarchy. Dominance hierarchies in Atlantic salmon have been little investigated in commercial-scale populations in sea-cages, and it is generally assumed that aggression in salmonids decreases with increasing density (Ellis et al. 2002). Social hierarchies are found in groups of adult salmon reared in tanks of 120 fish (15 kg $m^{-3}$, Cubitt et al. 2008), but the cost of establishing a dominance hierarchy in large populations is suggested to be too high in large groups such as the 50 - 400 000 salmon found in current farming practice (Oppedal et al. 2011). Instead, a type of scrambling competition for favourable space may exist in highly stocked sea-cages (Johansson 2007), where a new school structure could have emerged through some fish claiming the deeper spatial cage volume. Individual salmon in standard sea-cages with initial and final stocking densities of 6 and 15 kg $m^{-3}$ respectively displayed a wide range of coping styles, with a tendency for individuals with high growth rates to have less variation in body temperature, possibly through occupying the optimal water layer (Johansson et al. 2009) and excluding other fish from the best space in the cage.

Another possibility is that differences in individual coping abilities may have contributed to the observed patterns. Different individual coping styles has been suggested in Aqua Gen strain (same as in present study) (Kittilsen et al. 2009), and an alternative explanation is that

more proactive individuals (more active to prevent a stressor, Koolhaas et al. 1999) may have become more active to avoid the submergence situation than more reactive (flexible) individuals and sought the surface more often. The first dark night of submergence, four of the tagged fish in Sub42 stayed close to the roof and three of these individuals continued a reversed DVM or irregular diurnal vertical swimming pattern throughout the period, and they also had the lowest growth rates. The continuous swimming near the roof and lack of rest may have been energetically demanding. The more reactive individuals may have tolerated the submerged situation better, and managed to compensate sinking by a more normal DVM.

*Surface activity after re-surfacing*

Immediately after the submerged cages had been raised to the surface, an intense bout of surface activity ensued (Paper I). Rolling activity was particularly intense for the first 20 min and subsided gradually thereafter. On average two rolls seemed to be sufficient to re-fill the swim bladder, based on the rates of rolling per minute averaged over the 120 minutes after the fish gained access to the surface. This surface activity clearly demonstrated the need to refill the swim bladder after the submerged period.

*Skin erosions connected to submergence*

The shape and area of fins vary widely among species (Lindsey 1978), and fin condition is important for swimming ability. The fin condition of fish in wild populations is extremely rare in farmed fish (Hoyle et al. 2007), even when health and general condition are good. The relatively simple swimming challenges presented by living in tanks and sea-cages may lead to the development of a 'sufficient' status of the fins, compared to wild fishes' daily 'fight for life' which is likely to require high acceleration, fast turning and high manoeuvrability (Webb 1973). Intensively farmed fish receive fewer stimuli than their wild counterparts, where natural predators are absent and food is often in abundance, thus swimming challenges are probably simpler.

Snout and fin condition were negatively affected by the long-term, deep submergence in Sub42 in contrast to in Sub22 where no differences to controls were found. Occasional collisions were observed at night in Sub42, indicating that fish could be injured at night. 41 mature fish (25 control and 16 submerged) had fins with no sign of degradation (Korsøen unpublished data), suggesting that fin condition could be state-dependent and controlled by internal factors.

## Discussion of results – Atlantic cod

With its self-regulating swim bladder, the cod behaved quite differently in the submersible cages to the salmon (Paper IV). Prior to lifting and lowering, the average swimming tilt angle was close to horizontal (range: -3° to 1°) and swimming speed was low and variable (0.1-0.5 BL $sec^{-1}$). No schooling was observed such as in the salmon populations, but instead random and individually different swimming directions were prominent, which is more typical of a bottom-dwelling species like Atlantic cod (Rillahan et al. 2009). Cod often spends more than 90% of the time close to the bottom (Hobson et al. 2007; Moe et al. 2007).

### *Tolerance to positive buoyancy in cod*

Immediately after the ascents corresponding to a 40% pressure reduction, swimming speed approached critical swimming speeds (Gollock et al. 2009; Lurman et al. 2009) with head-down tail-up swimming tilt angles between -4° and -22° indicating that the cod were positively buoyant. The fish became more active after lifting events from shallow than from deeper depths. This may be explained by the inability to avoid the surface, as cod are pelagic but usually stay near the bottom (Godø and Michalsen 2000; Stensholt et al. 2002: Hobson et al. 2007), so that the surface may represent an unnatural high-risk area (Fernö et al. 1995).

Swimming activity, measured as swimming speeds, was in general less at the lower temperature, but the water temperature did not directly affect the response to lifting. Swimming speeds close to the critical speed indicated that a 40% pressure reduction was close to the limit for behavioural coping by swimming, and therefore a relatively high

immediate stress situation, most likely a secondary response with increased metabolism and ventilation.

Recovery time was independent of start depth prior to the 40% pressure reduction ascent (Fig. 6 in Paper IV), with total recovery taking 8-10 h. Loss of behavioural control was never observed, and the return of feeding activity after 2 h indicated that this situation was not a major stressor for the cod to cope with. No difference in recovery time between the low- and high temperature experiments was seen, although swimming speeds was generally less under the low-temperature regime.

This indicated that the gas transport from the swim bladder via the oval and blood to the gills was independent of temperature, which is in contrast to the model put forward by Strand et al. (2005). The model also suggested a short time (2 h) for recovery at high water temperature compared to the present results (8 h). Therefore, care must be taken in using this model to estimate recovery time after ascents for farmed cod in crowded sea-cages, especially in warmer water (16°C). The experimental results highlight the importance of ground-truthing such physiological models in realistic aquaculture settings and environments before their outputs are applied.

*Pilot test – a "lifting accident"*

After the experiment was finished, a pilot test was done to simulate a "lifting accident", where a 54% pressure reduction step (14-1 m) was performed after only 6 h of recovery following a 30-14 m step. This resulted in intense downward swimming during the first few minutes, after which several cod gradually turned and swam sideways, rising to the net-roof. After 20 minutes, 18 of 100 cod were floating belly-up underneath the net-roof. The cage was lowered again to 14 m, where the fish recovered within 90 minutes, with several resting on the tight net-floor. This was clearly a situation in which the pressure reduction exceeded the limit for coping by compensatory swimming, but at the same time a pressure reduction that was not sufficient to rupture the swim bladder, a potential safety-valve to regain behaviour control (Love 1980).

*Tolerance of negative buoyancy in cod*

Rapid lowering caused negative buoyancy, as the increased pressure compresses the soft-walled swim bladder (Paper IV). According to Boyle's Law, the swim bladder was reduced from 5% of body volume to 2.5%, 1.7%, and 1.3% after descents from the surface to 10, 20 and 30 m. To avoid sinking while the gas gland secretes gas into the swim bladder, the cod increased swimming speeds by factors of 1.3 to 2.3 immediately after the cage was lowered, and swam at an average 30º head-up tail-down angle. Neither pressure level nor temperature affected this immediate response, and appetite was less affected than after the lift events. More than 50% of the fish rested on the tight net-bottom after 1.5 h at low temperature and after 4 h at high temperature (Paper IV).

This resting option may have helped the cod to recover to neutral buoyancy within 18-90 h, a period that was dependent on pressure and temperature. Without this possibility, constant swimming might have raised the stress level and reduced the capacity for gas secretion, resulting in delayed depth adaptation.

Gas secretion in cod is reported to occur 5.5 times faster at 17°C than at 0°C (Harden Jones and Scholes 1985; Strand et al. 2005). The present findings correlate well with these estimates at 5°C, but at 16°C the recovery time after lowering was about twice as long as the model prediction. Optimal predicted swimming tilt angle of 7° (Strand et al. 2005) also differed from the observed 16-25° swimming tilt angle during recovery in my experiments.

*Possible reasons for deviations between actual recovery time and model estimates*

The conditions in the sea-cage probably differed from the conditions in the pressure chambers on which the model by Strand et al. (2005) was based (Harden Jones and Scholes 1985), likely being more naturally for the free swimming cod in the present study, and the swimming repertoire led to a clearer picture of the total period needed for absolute recovery.

Alternatively, it is possible that farmed cod have a lower gas-exchange capacity than the wild cod used in the pressure chambers by Harden Jones and Scholes (1985). Farmed cod are restricted to relatively limited depths (0-3 m in tanks and 0-50 m in sea-cages) throughout

their entire life cycle compared to wild cod, which range between depths of 0 to around 500 m (Godø and Michalsen 2000; Stensholt et al. 2002; Hobson et al. 2007). The conditions in the tanks during their juvenile stage and shallow sea-cages may not have permitted farmed cod to adapt to a new and unpredictable environment in a large sea-cage and their behavioural repertoire may not be sufficiently developed to successfully adapt to changing water pressure. Furthermore, livers of farmed cod are larger (13-15% of body weight (Paper IV and Gildberg 2004) than wild cod (4.5-9.5%, Gildberg 2004) and can provide extra lift. Both of these factors may have resulted in less active gas-exchange and therefore impaired coping ability. Comparative studies of behaviour during vertical steps of wild and farmed cod, in combination with studies of the size of the gas gland, may reveal differences in gas exchange capacity. If a difference does exist, there could be a potential for conditioning and habituating farmed cod to increase gas exchange capacity to enhance buoyancy regulation.

## CONCLUSIONS AND PERSPECTIVES FOR THE FUTURE

Adaptation to the ambient water pressure is essential for the welfare of Atlantic salmon and cod. A state that deviates from neutral buoyancy will lead to various degrees of stress. Fish have a wide anatomical, physiological and behavioural repertoire to cope with vertical migration according to the depth range that they inhabit. The functions of the swim bladder in physostome and physoclist populations are therefore very different. The Atlantic salmon is a surface-dwelling species that in the wild often dives rapidly and deeply and has a simple mechanism for emptying and re-filling the swim bladder, while Atlantic cod, as a demersal species with a more advanced and slower mechanism for adjusting the amount of gas in the swim bladder, needs more time for vertical migrations.

The studies described in this thesis have demonstrated that short-term shallow submergence of Atlantic salmon in large sea-cages did not have significant negative consequences if lighting was provided to permit the fish to implement a compensatory swimming speed throughout the day and night (Papers I and III). Deeper long-term submergence in winter with long dark nights, on the other hand, had several physiological and behavioural negative consequences for the salmon (Papers II and III).

Submergence of healthy Atlantic salmon with the net roof at 4 m can be done for shorter periods < 2 weeks provided sufficient lighting to maintain compensatory swimming speed at day and night. Ascent to the surface and access to air must be provided at least until the surface activity normalise before any subsequent submergence.

Whether long-term submergence of Atlantic salmon in sea-cages equipped with an artificial surface (air - dome) can be successful and not compromise growth or welfare requires further investigation. Decompression stops may be needed during the lifting operation, as the rate of gas expulsion from the swim bladder in salmonids has not been clearly described. Other unknown factors such as gas embolism may also contribute to poorer performance and welfare.

Atlantic cod managed to regain neutral buoyancy at all depths to which they were vertically moved (Paper IV). The high swimming activity indicated that a 40% pressure reduction is near the upper limit for a safe lifting step in this species. Rapid lifting events were more challenging when the cod ascended close to the surface. Independent of water temperature (5 and 16°C), cod needed around 8-10 h to recover neutral buoyancy after lift events from five different start depths (ranging from 30 to 8 m).

Cage lowering was less challenging for cod, but this probably depends on whether cod can rest on the net-bottom. A compressed swim bladder after descents from the surface to 10-30 m leads to negative buoyancy, which required 18-90 h to re-fill by gas secretion, which is a temperature-related process. Crowding stress in a highly stocked sea-cage in combination with constant swimming may reduce gas secretion capacity.

Submersible cages are likely to be good for Atlantic cod in several ways. Compared to deep, open-to-the-surface nets, a submersible cage- roof may secure uncontrolled ascents. Less stress will probably occur when cod have no contact with the surface. Cage designs for bottom-dwelling species like Atlantic cod that permit resting might improve growth performance and welfare. Other factors such as the degree of early sexual maturation may be reduced in a submerged cage with more efficient manipulation of day length by artificial lighting as the sunlight intensity decreases rapidly with increasing depth.

Other physostome and physoclist species cultured in submersible cages will face similar buoyancy regulation challenges as Atlantic salmon and cod. Fish farmers should follow safe protocols for pressure changes and acclimation time, based on knowledge of each species. The methodology used throughout this thesis, in which behaviour was used as a proxy indicator to detect the influence of positive and negative buoyancy, can be utilised to identify appropriate interval steps for cage ascents - and descents, and appropriate submergence durations for other existing and emerging species in aquaculture.

The basic idea behind submerged fish farming is to adapt to exposed conditions and rough sea states, and to farm fish at depth, rather than in the surface layer (Ryan 2004). The cost of constructing a surface cage, designed for extreme conditions, would be high. At small scales, several submerged farming systems work (e.g. Benetti et al. 2010), but large-scale submersible cages and equipment that better incorporate the requirements of operations that need to be made both daily and at longer intervals still need to be developed (Ryan 2004).

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